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Parietal eye of the lizard: Neuronal photoresponses and feedback from the pineal gland

(neurotransmitter/photoperiod/5-hydroxytryptamine/norepinephrine)

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ABSTRACT **The parietal eye of the lizard responds to illumination by sending afferent impulses to the pineal gland during daylight, the photophase. The pineal gland has efferently conducting neurons which are especially sensitive to norepinephrine and whose feedback to the parietal eye enhances its photo responsiveness. During the scotophase, at night, the eye generates afferent impulses to the cessation of light and the pineal efferents are most sensitive to serotonin. Thus, the photo- and chemoresponses of this system of interacting neurons are nearly reversed during the two phases of the daily photoperiod of the lizard.**

The parietal eye in the lizard is connected with the central nervous system and the pineal gland by the small parietal nerve (diameter, 15–25 μm). The parietal nerve is composed of 200 to 600 axons, most of which are unmyelinated (1, 2). Not only does the parietal eye respond to illumination with a characteristic electroretinogram (3), but its sensitivity varies with the wavelength of the stimulating light (4, 5). However, neuronal impulses had not been detected within the parietal nerve of any lizard. While most workers held that this nerve did, in fact, conduct impulses, such a position was not unanimous (6).

We have developed a preparation (Fig. 1A) for recording impulses conducted by the parietal nerve of the collared lizard, *Crotaphytus collaris*§. Impulse activity was recorded with a suction electrode attached to the side of an intact parietal nerve, and light levels were simultaneously monitored with a photocell. We conducted the first series of experiments in early afternoon during the photophase of the lizards, and detected small (15–35 μV) impulses. When the eye is illuminated, this impulse activity increases sharply, as shown by Fig. 1B*1*. This is a typical "on" response, having both phasic and tonic impulse components with an average onset latency of about 350 msec. In the impulse histogram of Fig. 2A, it can be seen that the ongoing activity in the nerve is increased more than 4-fold by illuminating the parietal eye. We did not regularly detect any increase in activity to the cessation of light (an "off" response) under these experimental conditions.

The parietal nerve was transected, and recordings were taken from each side of the cut in order to insure that these impulses were generated by the eye and not the pineal gland. Action potentials were again recorded in response to illumination, but only from the parietal eye (distal) end of the cut nerve and never from the proximal end. These action potentials were indistinguishable from those of the intact nerve. Clearly, then, the parietal eye responds to light with an afferent neuronal response.

In recording from the pineal (proximal) end of the cut nerve, we detected no neuronal responses whatsoever to illumination of either the pineal gland or the parietal eye. To insure that this silent proximal nerve was still capable of impulse conduction, we used a blunt glass rod to probe the pineal gland, and generated bursts of impulses which traveled efferently toward the eye. The region of mechanical sensitivity of the pineal appeared to be localized near the parietal nerve. Pineo-parietal efferent neurons have also been detected by electrical stimulation experiments on frogs (7). Notwithstanding this observation, an efferent function for mechano-reception by the pineal gland is not immediately obvious. We surmised that the pineal efferent neurons might be sensitive to those neurochemicals that abound within the gland itself (8–11): norepinephrine and 5-hydroxytryptamine (or serotonin). Therefore, we performed experiments designed to measure any responsivity of the neurons to these putative neurotransmitters by ejecting approximately 0.05 ml of a solution (1 mg of transmitter per ml of lizard saline, or about 50 μg of transmitter) from a syringe onto the pineal gland. Controls consisted of vigorous jets of saline without neurochemicals. These controls were without effect, but a brief application of 5-hydroxytryptamine onto the gland

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§ The physiological saline for this terrestrial lizard is a Tyrode's solution that has an increased tonicity with these millimolar salt concentrations: NaCl, 145; KCl, 2.7; $CaCl_2$, 2.7; $NaHCO_3$, 11.9; and $NaHPO_4$, 0.3. Lizards that had been maintained under natural photoperiod were decapitated. After the lower jaw was removed, the brain was dissected from the ventral surface producing a preparation consisting of the parietal eye, nerve, and pineal gland within the cranial cavity. This preparation was pinned ventral side up to a layer of Sylgard (Dow Chemical) resin in a glass-bottomed chamber. The preparation could then be illuminated either laterally or dorsally through the transparent resin with a substage darkfield condenser. Light sources were 6V focusable microscope lamps powered by storage batteries, and light duration was controlled by mechanical or electrical interruption. The illumination level was monitored directly above the preparation with a silicon photocell whose voltaic output was amplified and displayed upon an oscilloscope trace. Suction electrodes were attached *en passant* to the parietal nerve with negative pressure, and their active and reference leads provided the differential input to a Grass P15 AC Preamplifier. A narrow acceptance window was usually established with the high and low pass filters set at half-amplitudes at 30 and 300 Hz, respectively. The output was amplified an additional 100× with a Barrows Operational Amplifier Manifold which fed into a Tektronix D 10 Oscilloscope in parallel with a Grass Audio Monitor. This monitor was important in successful electrode placement, for even in those preparations in which the nerve was too small to be seen, it could be located by listening for activity after teasing away the meninges near the parietal vein and pulling the presumptive nerve into the electrode tip. Permanent records were taken from the face of the oscilloscope with a Grass Kymograph camera.

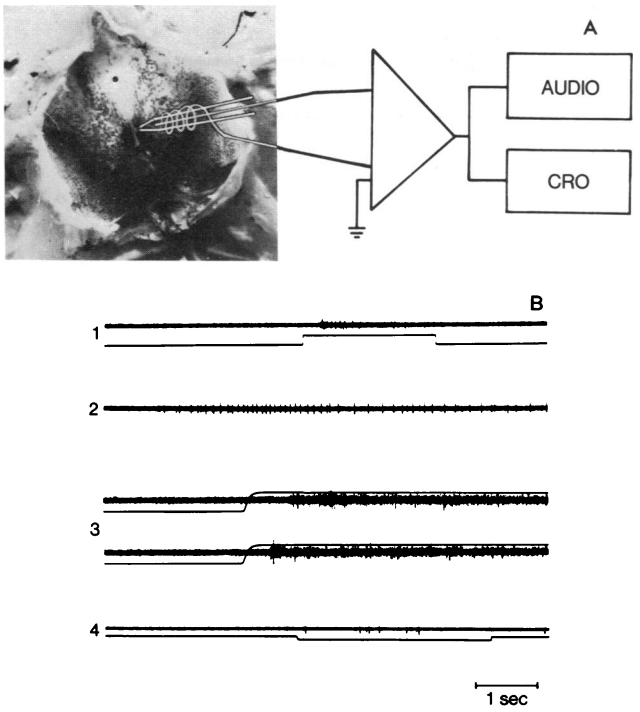

FIG. 1 (A) A ventral view of the parietal eye-pineal gland complex within the cranium of the collared lizard *Crotaphytus collaris* with the brain removed. The suction electrode and major electronic components are diagrammatically illustrated. Within the cranium, the parietal eye is the dark spherule which is haloed by the light passing through the fenestra of the parietal bone. The parietal nerve connects the eye and pineal gland (lower center). (B) *1*: Afferent impulse activity of the parietal nerve (top trace) during a pulse of light (bottom trace, upward deflection = light on). Recording from a preparation in photophase showing both phasic and tonic impulse responses to illumination. *2*: Efferent impulse activity in the parietal nerve in response to a brief application of serotonin onto the pineal gland (application at the beginning of the trace). *3*: The effect of the norepinephrine-induced burst upon the impulse response of the parietal eye to light stimulation. The top pair of traces is a baseline control which shows the "on" response of the eye when illuminated (by turning on lamp). The bottom pair of traces shows the response of the same preparation to an identical photostimulation immediately after a chemically stimulated efferent burst of impulses from the pineal gland. After efferent activity the latency of the "on" response is halved, and different units now fire in response to illumination. *4*: The "off" response of the parietal eye recorded from a lizard during its scotophase.

doubled or tripled the efferent impulse activity in the parietal nerve, as can be clearly seen in Fig. 1B2. The 5-hydroxytryptamine effect is depicted by the impulse histogram of Fig. 2B, while Fig. 2C illustrates the effect of norepinephrine application. The response to norepinephrine is a dramatic 4-fold increase in impulse activity that falls off more or less exponentially, but remains above control levels for about 30 sec. As an additional control, we recorded from the parietal end of the cut nerve once more and attempted to stimulate the eye with neurochemicals: the parietal eye did not respond to either 5-hydroxytryptamine or norepinephrine.

The afferent activity of the parietal nerve is apparently characterized by photoresponsiveness, whereas, the efferent activity of the pineal gland is characterized by chemoresponsiveness. This exclusive dichotomy in responsivity provided us with an experimental control by which we could measure any efferent influences upon peripheral photoreception. When recording from an intact nerve, a light pulse onto the parietal eye generated an afferent "on" response (Fig. 1B3, top traces) which served as baseline control. Norepinephrine was then ejected onto the gland and induced a characteristic burst of efferent neuronal impulses. After about 40 sec, when the activity had returned to spontaneous levels, the eye was reilluminated with an identical light pulse. This second illumination generated an "on" response that had distinctively different afferent units (usually larger) and whose latency was about half that of the control response (Fig. 1B3, lower traces). This efferently induced increase in excitability persists for 2–3 min after a single norepinephrine jet. When two exposures to light, without norepinephrine stimulation, are separated by an interval of less than 1 min, there is usually a reduction in impulse activity to the second exposure (habituation). Thus, the augmented photoresponsiveness following an efferent burst may be even greater than these results show, as it is superimposed upon the habituated response. We conclude, then, that the norepinephrine-sensitive efferent neurons feed back onto the parietal eye and function in augmenting the responsiveness of the eye to light. We saw no such effect with serotonin.

The pineal gland is well known (8–11) for circadian rhythmicity in the concentrations of its endogenous neurochemicals. Hence, we suspected that the pattern of neuronal responsiveness to light and to chemicals might be affected by the photoperiod of the lizard. We repeated the entire experimental paradigm during the scotophase of the lizards. The results of this procedure are depicted in the right column of Fig. 2 (D, E, and F). Illumination of the parietal eye at night does not detectably increase the activity in the nerve (Fig. 2D). However, the cessation of the light stimulus usually produces an increased impulse activity—an "off" response with an average latency of about 270 msec (Fig. 1B4). The "off" response is more variable than the "on" response, and a strong "off" response often precludes a second "off" response when the interstimulus intervals are less than 1 min.

Fig. 2E and F illustrates the effects of chemical stimulation upon the pineal gland efferents during scotophase. Serotonin (Fig. 2E), which had but a small excitatory effect during photophase, produces a drastic increase in impulse activity: about 100 times greater than that of controls. This increased activity, as that of norepinephrine in photophase, continues for about 30 sec. The rapid decrease in activity might come about by either a desensitization of the receptor or a diffusion of neurochemicals from the pineal gland. The latter alternative is more plausible, since two to three sequential chemostimulations, each separated by intervals of about 5 min, generated bursts whose impulse frequencies were very similar. The norepinephrine response (Fig. 2F), so dramatic in photophase, consists of but a short phasic burst in scotophase. Thus, the pattern of photoresponses by parietal eye afferents and chemoresponses by pineal gland efferents in a scotophase preparation appears to be the converse of that seen in a photophase preparation.

This interacting system of photo- and chemosensitive neurons is not in one mode during daylight and the precise converse at night. At least two differences exist. First, the impulse activity of the chemosensitive, efferent neurons had no obvious effects upon the photoresponses of the eye at night. Second, the parietal eye responds to a jet of 5-hydroxytryptamine at night with a long burst of impulses.

We suspect that the alternations between the "on" and "off" responses by the parietal eye are due to variations in levels of pineal gland feedback. The alternation between

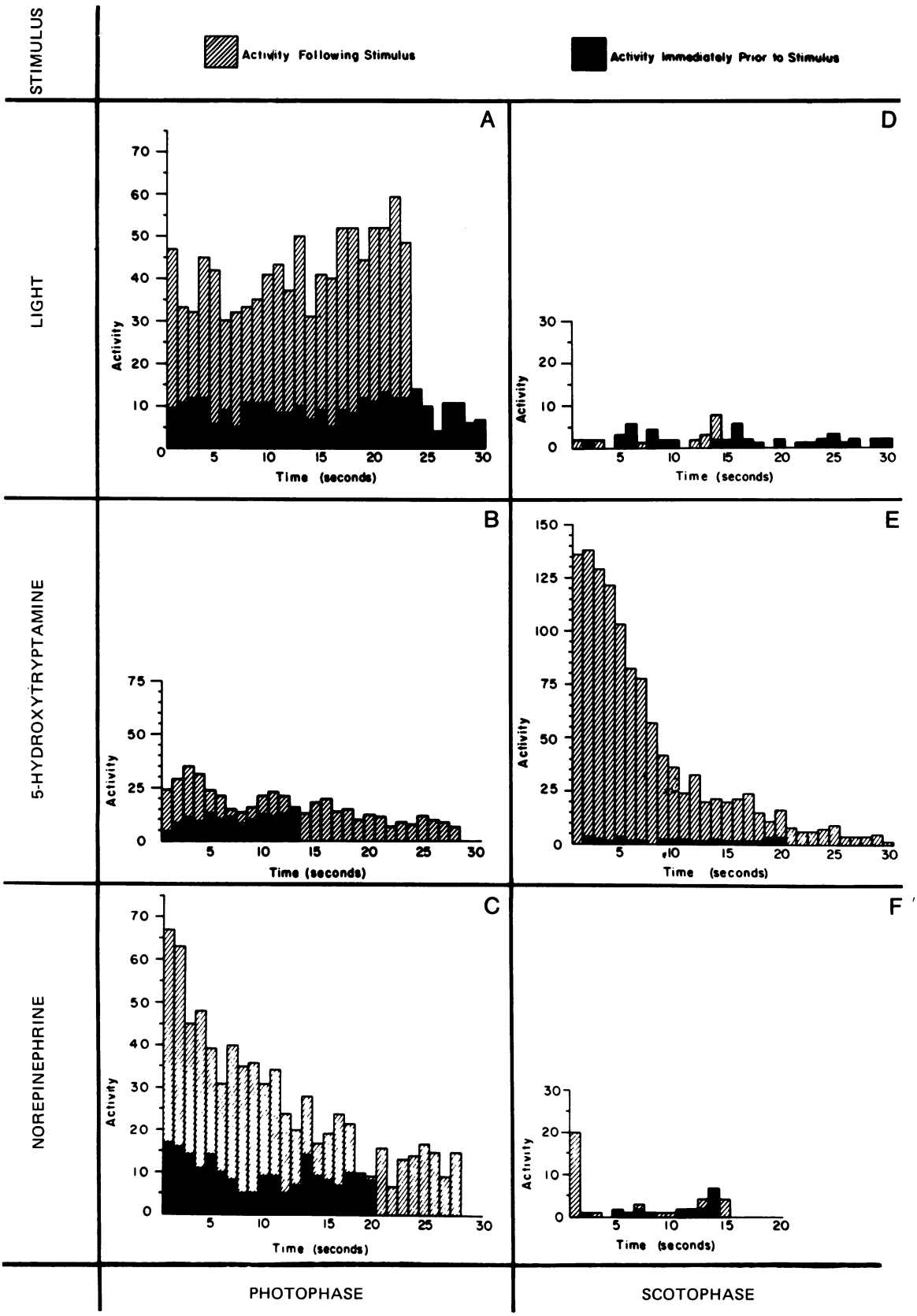

FIG. 2. Histograms of the impulse activity within the parietal nerve, immediately before and after stimulation of the parietal eye-pineal gland complex removed from lizards in photophase (A, B, and C) and in scotophase (D, E, and F). A and D illustrate the increase in afferent activity generated by illuminating the parietal eye. B and E show the generation of efferent activity by stimulating the pineal gland with 5-hydroxytryptamine (note the reduction of the activity scale in these 5-hydroxytryptamine histograms). C and F illustrate the efferent activity emanating from the pineal gland after stimulation with a solution of norepinephrine.

norepinephrine and 5-hydroxytryptamine sensitivity is the probable mechanism by which feedback is controlled. The endogenous oscillations of neurochemicals within the pineal gland itself could underlie the alternating chemosensitivities of the efferent neurons. If this were the case, we should expect to find neurons whose response characteristics are a function of photoperiod, and that is what seems to prevail.

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